STUDIES ON CHEMICAL CHANGES DURING THE LIFE CYCLE OF THE TENT CATERPILLAR (MALACOSOMA AMERICANA FAB.). II. NITROGEN AND ITS RELATION TO MOISTURE AND FAT[1]

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If fats and salts are removed from living matter a mass of insoluble organic material is left behind. Upon analyzing this material we find roughly 50 per cent. carbon, 22 per cent. oxygen, 7 per cent. hydrogen, 0.3 to 1 per cent. sulfur, some phosphorus and traces of other elements, and about 16 per cent. nitrogen. This nitrogenous material (proteins) plays a predominant role in vital phenomena. The proteins are decomposed into amino-acids and distributed into the blood. Some of the amino-acids present in the digestive tract are attacked by bacteria and changed into ammonia and carbon residues. In metabolic processes amino-acids can be changed to sugars. Nitrogen determinations can be regarded as important since it is assumed that the insect uses nitrogen to form protein material in the tissues.

It is well known in physiological chemistry that proteins laid down in a cell for storage checks the metabolism of such a cell. Off hand, therefore, one would expect to find no storage of protein material in active cells. In the case of insects one might suppose that nitrogenous material would be stored in the larvæ, possibly in special non-active cells, to be used in metamorphic processes. This is not conceivable in a rapidly growing larva, where all cells would seem to be needed for activities. Aside from the energy necessary to sustain life, the larvæ are rapidly gaining in length and weight and have also to produce a new skin several times. In the mammalian body fats are more avail-

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able for energy than proteins and only when insufficient amounts of fats and carbohydrates are present proteins are used. However, this does not necessarily mean that the same is true for insects.

Methods and Material

The same material previously described (8) has been used. Total nitrogen was determined by the Kjeldahl method.

Results

Analysis of the egg masses shows that shortly after deposition, when larvæ are formed, from 50 to 53 per cent. of the total dry weight is due to the nitrogenous cover and egg cases. This percentage changes with the age of the egg masses. The cover contains 8.0 to 8.4 per cent. moisture, and about 16 per cent. nitrogen and no fat. The moisture of the egg shells with as much of the gelatinous cover removed as possible appeared to be about 15.7 per cent., while the moisture of the larvæ taken out of the egg cases averaged about 60 per cent. The average nitrogen content

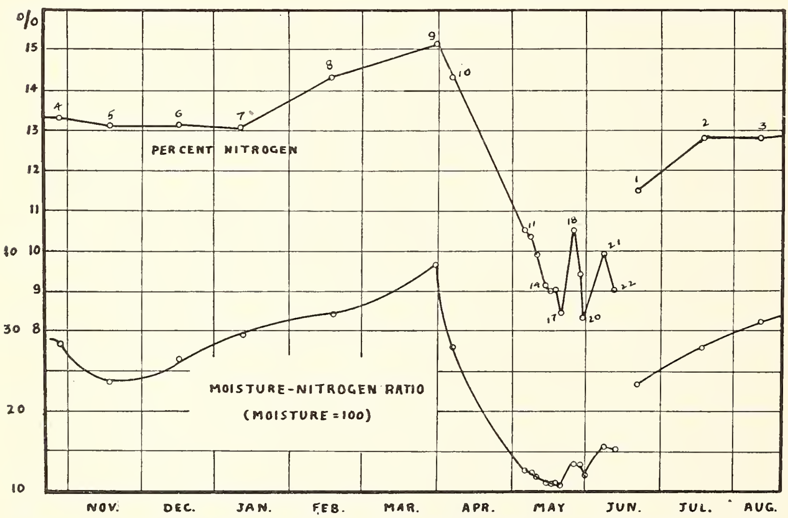

Fig. 1. Total nitrogen content of egg masses, larvæ, pupa and adults and nitrogen-moisture ratios. Nos. 1–8, egg masses; no. 9, newly hatched larvæ; nos. 10–17, growing larvæ; no. 18, full grown; no. 19, prepupal; no. 20, just pupated; no. 21, pupæ ready to hatch; no. 22, adults.

of the total egg masses from the time they were deposited to the hatching of the larvæ was about 13 per cent. Figure 1 shows the fluctuation in total nitrogen content during the entire life cycle of the insect. It can be seen that total nitrogen seemed to increase rapidly during the period when the larvæ were forming inside the egg cases. It has been found (8) that during this time fats decreased rapidly. From July until January the nitrogen content decreased slightly, coinciding with the slight decrease in fatty substances. There seems to be a rise of nitrogen again just before hatching, which again corresponds with a rapid decrease of the remaining fat (reduction of nearly 50 per cent. of the total fat present). At the time of hatching the nitrogen content of the young larvæ was 15.1 per cent. As soon as active feeding began the total nitrogen dropped apparently considerably. As has been pointed out before (8) at least one fourth of the total wet weight of the caterpillar was due to gut contents. The droppings contained on an average 2.5 per cent. nitrogen and 70 per cent. moisture, so that the actual nitrogen content of the caterpillar bodies was greater than indicated by the figures in the graph. For example a batch of full grown, but actively feeding caterpillars, contained 9.0 per cent. N, including gut contents, while the droppings analyzed about 2.5 per cent. N on a dry basis. Caterpillars starved for 40 hours contained 8.45 per cent. nitrogen. Calculations, on the basis of no droppings present, show that the bodies should contain about 10.6 per cent. nitrogen. This amount practically corresponds to the amount of nitrogen found when the insects stopped feeding (10.55 per cent. N) and it seems fair to conclude that from 1.5 per cent. to 2.0 per cent. of nitrogen should be added to the figures of the growing caterpillars in order to derive at the proper nitrogen contents for their bodies. As stated above the newly hatched caterpillar contained 15.1 per cent. N (dry weight) while the growing larvæ decreased rapidly to 12.5 and finally to 10.5 per cent. (adding 2 per cent. N for the difference of gut contents); this leaves a difference from 2.5 to 4.5 per cent. N on the basis of dry weight or a decrease of from 18 to 30 per cent. nitrogen of the original amount present. These figures show thus that the body weight of the growing caterpillars increased at a greater rate than its nitrogen content. This is partly accounted for by the fact that large quantities of fats were stored during this period. However, if we examine the curve, we see that during the last instar nitrogen increased again and it might be assumed that a part of the nitrogen intake went into the makeup of the skins of the growing caterpillar which were shed at intervals. Only one analysis of discarded skins is available but this shows that they contained 12.2 per cent. nitrogen. Since no actual weights of all discarded skins are available it is impossible to calculate the total amounts of nitrogen used for their construction. From the time the larvæ made ready for pupation (becoming flabby) until they had just pupated insects lost actually 2.29 per cent. nitrogen or 22.6 per cent. of the total nitrogen present at the first stage. The cocoons contained 16.2 per cent. nitrogen, with a moisture content of 10.0 per cent. It is interesting to note that from the time no food was taken until the prepupal stage 1.2 per cent. N was lost, corresponding to 11.2 per cent. of the total present or exactly half the loss sustained until they had just pupated. Since the actual loss was 22.6 per cent. and 16.2 per cent. was recovered in the cocoons we might assume that part of the difference was lost during this reconstruction period. From the fact that during this period nitrogen decreased and fatty materials actually increased the conclusion might be drawn that nitrogenous materials only were used during this part of the metamorphosis. However, this does not complete the story. During the period of ''just pupated'' to ''ready to hatch'' the nitrogen content increased with 1.63 per cent. of the total dry weight or 19.7 per cent. of the total nitrogen at the first stage. At the same time the fatty substances decreased with 2.81 per cent. or nearly 10 per cent. of the total amount present. It would seem therefore that during this second period metabolic processes necessary for the maintenance of life were continued at the cost of fatty substances. Alderhalden (1) found that nitrogen increases considerably with pupation.[2]

The relation of the percentage of nitrogen to the percentage of fats present during the life cycle is presented in figure 2. The apparent increase of nitrogen during the first and last stages of

[2] See also note 3.

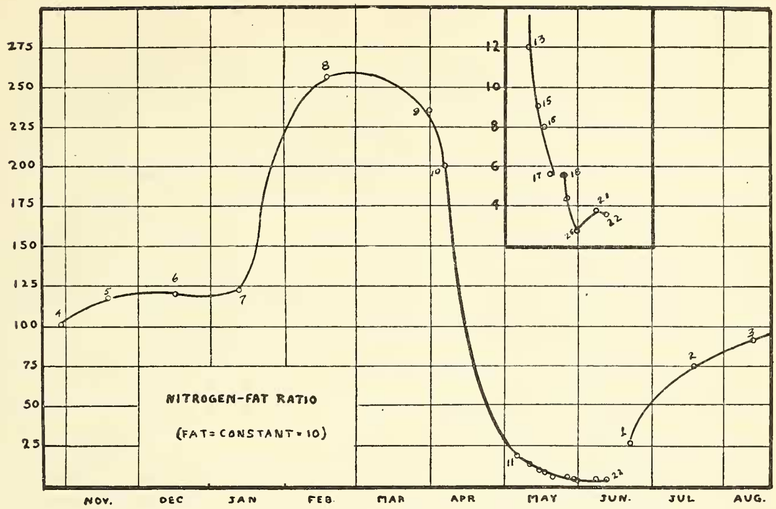

FIG. 2. Relation of nitrogen to fat present in tent caterpillar during entire life cycle. Nos. 1–8, egg masses; nos. 9–18, larvæ; no. 19, prepupæ; nos. 20–21, pupæ; no. 22, adults. Nitrogen calculated to a constant fat basis of 10.

the larvæ in the egg masses is here accentuated since fat decreased rapidly. There is no doubt that vital activities were carried on during this period through the oxidation of the fatty substances. Fat being the most prolific source of energy accounts for this. Since fat increased rapidly during the growing period of the caterpillars, the decrease in nitrogen was here also accentuated. The rate of decrease and increase of nitrogen during pupation is by no means the same as during the two other stages. In the last part of the curve the rate appears to be nearly equal, but the inset (showing a part of the life cycle) indicates that there were differences.

Kellner (3) found that the larvæ of the silk worm contained 12.0 per cent. nitrogen upon hatching which was reduced to 9.75 per cent. in the last instar. One analysis made on the pupa showed 9.16 per cent N and one on the adult 9.49 per cent. N. It can be seen that these figures correspond in a general way with my results. There was only one analysis made during each instar, and one each of pupæ and adults. This might explain why variations during certain stages escaped unnoticed by this investigator.[3]

Nitrogen fluctuations in relation to moisture content (figure 1) and to fat (figure 2) seem to make a chemical division of the life cycle in at least 6 parts permissible, namely (1) formation of larvæ in egg cases, (2) rest period of larvæ in egg cases, (3) growing larvæ, (4) reconstruction stage of larvæ into pupæ, (5) rest period of pupæ, (6) adult stage. Two of these periods would be unsuitable for the application of control measures and the best would appear to be when metabolic processes are most active, or during the period shortly after hatching.

The growing caterpillars decreased in total nitrogen in spite of the fact that they were continuously eating material containing a certain amount of nitrogen. Analysis of leaves on which the caterpillars were feeding showed that they contained from 3.0 to 3.9 per cent. N (dry basis) and from 65 to 72 per cent. moisture. Their droppings contained about 2.5 per cent. nitrogen. How much nitrogenous material, or how much actual nitrogen was consumed is unknown to me since no measurements of the amount of food consumed were taken. A good deal of the nitrogenous materials present in the leaves are not available but it is clear that the digestive juices extracted from 0.5 to 1.5 per cent. of the nitrogen present. In this process the carbohydrates in the

[3] After this paper was prepared for publication a reprint of an article "On the Biochemistry of the Wild Silk Worm," published in the Memoirs of the Coll. of Sci., Kyoto Imp. Univ., v. 9, 1925, by O. Shinoda, came to hand. This investigator found a nitrogen content of the larvæ varying between 1.3 to 1.0 per cent. (apparently on a wet basis). From the fourth molting until after cocooning he found to his "astonishment the nitrogen content increased from 1.2 per cent. to 2.6 per cent., *i.e.*, 9.2 per cent. to 10.4 per cent., when calculated in dry materials." His figures show a further increase during pupation. If nos. 19 and 21 are compared it may be seen that this corresponds to my findings. However, I found that nitrogen increased rapidly after the insect stopped feeding with an increase in the prepupal stage, then a decrease and again followed by an increase, with the result that actually more nitrogen was present in the insects when they were ready to emerge than when they were still feeding during the last instar. Apparently Shinoda missed the period when nitrogen decreased during metamorphosis. Otherwise his figures agree remarkably well with my results.

leaves, which are used especially for oxidation and the formation of fatty substances, are taken in at the same time.

The egg masses increased apparently in total nitrogen in spite of the fact that through handling a small part of the cover, high in nitrogen, was lost. There are three reasons for this apparent increase in nitrogen percentage: (1) Fat decrease at greater rate than nitrogen, (2) Carbohydrate material decrease at greater rate than nitrogen, (3) Synthesis of protein decomposition products into simple proteins thereby preventing loss of nitrogen. In order to gain some insight into the activities and decomposition processes, qualitative analyses were made of the nitrogenous cover, larvæ taken out of the egg cases and whole egg masses for ammonia nitrogen, albuminoid nitrogen, total amino-nitrogen, amino-acids, glycogen and sugars. These determinations were repeated at intervals throughout the life cycle and will be presented more in detail later. Table 1 shows some of the results obtained on the nitrogenous cover and egg masses.

In the animal body simple proteins are distributed into the blood largely in the form of amino-acids. The circulation of blood is so rapid that the amino-acids are removed almost as rapidly as they enter, so that there is no accumulation of amino-acids in the blood, but non-protein nitrogen (amino-acids, urea, ammonia, etc.) always occurs. The question whether or not similar products are present in the insect body is of interest. Muttowski (6) reports finding in insects' blood ammonia, gelatin, a nuclea protein and at feeding possibly various hydrolyzed proteins, aside from a number of metallic bases and pigments. He also tested a number of insects (5) for copper and the finding was "interpreted as forming the nucleus of another respiratory pigment, hemocyanin," not as yet demonstrated. The results presented in table 1 are on egg masses and there was thus no active feeding. The determinations were made on egg masses from which the nitrogenous cover was removed as much as possible. The total nitrogen in the case of the cover indicates that it consisted practically all of nitrogenous matter, a trace of sulfates, some carbonates and insoluble ash being present. Amino-nitrogen, determined by the Van Slyke method, includes urea. In considering first the analyses of the cover a certain amount of

TABLE 1.

ANALYSES OF EGG MASS AND NITROGENOUS COVER*
(NOVEMBER 17, 1924).

	Egg mass	Cover
Total nitrogen	10.04 per cent.	16.15 per cent.
Amino-nitrogen	1.426 per cent.	0.835 per cent.
Ammonia-nitrogen	13.4 ppm	35.4 ppm
Albuminoid-nitrogen	669 ppm	1062 ppm
Urea	++++	++
Amino-acid reaction:		
Tryptophane	++	++
Xanthoproteic (Tyrosine, Phenylalanine and tryptophane)	++	−
Ehrlich's diazo: (Histidine, tryosine)	−	−
Millon's: (organic compounds containing a monohydroxybenzene nucleus)	−	−
Cholesterol	−	−
Reduced sulfur (cystine, cysteine)	+	±
Carbohydrates (Molisch reaction)	++++	+
Sugars	++	−
Glycogen	3.2 per cent.	+
Ether soluble (fats)	1.20 per cent.	0.0 per cent.
Phosphorus (P_2O_5)	++	+

* ppm = parts per million; − = negative; + = positive.

amino-nitrogen is recorded. The weight of amino-acids from a given weight of protein is greater than that of the protein from which they are derived. Decomposition of the proteins is hydrolytic or in other words water has been taken up. No great energy transformations accompanies the decomposition. During the process of digestion and absorption of proteins in the animal body amounts of ammonia are set free. Tissues can also decompose their protein and amino-acids, namely when not sufficient amounts of carbohydrates are present to supply energy. In the latter case organic acids are formed plus urea. In the third place protein decomposition takes place readily outside the animal body, through the agency of bacteria. We note that ammonia was present in the cover. The presence of both amino-acids and ammonia seems to indicate therefore that the cover was slowly decomposing. Turning to the analyses of the egg masses we note a considerable amount of amino-acids being present. It is likely that a good deal of it was in reality urea. Probably three amino-acids were present, namely: Tryptophane, phenylalanine and cystine or (and) cysteine. The xanthoproteic reaction gives the presence of tryosine, phenylalanine and tryptophane. Tryptophane was found with its special reagent, tyrosin was absent with Ehrlich's diazo reagent, while the cover did not show positive results with the xanthoproteic reagent, it can be deduced that phenyl-alanine was present in addition to tryptophane. However, the presence of phenylalanine was not demonstrated at that time. Roose (7) demonstrated the presence of this amino-acid in the cocoon. Tryptophane is a necessary constituent of food. If it is remembered that not protein but amino-acids are required by the body, it seems significant that tryptophane was present in the resting larvæ. Since tryptophane is formed in tryptic digestion it might have been formed from simple protein-matter and this would explain the reduction of the total nitrogen. During the stage that nitrogen content remained stationary the life of the insect in the egg case is one of existence rather than living. Since nitrogen was reduced rapidly in the growing caterpillar the difference in activities between the two stages is obvious. During the third stage in the life of the encased caterpillar an apparent increase in nitrogen was noticed together with a rapid decrease of fats. It is clear that fats were used for energy, but from where did the increase in nitrogen come? It is a bold hypothesis to assume that the decomposing cover of the egg mass furnished the caterpillar with ammonia and amino-acids for resynthesis of protein matter. In a next article this whole question will be discussed more in detail in relation to the data on ash and its constituents.

As has been pointed out above tyrosine was not present in the encased larvæ. Tyrosine, one of the first discovered end products of protein decomposition, is present in plants and animal tissues. It is needed by mammals but they are unable to make sufficient to supply their needs. It is formed in tryptic digestion (from zein, found in maiz for instance) and when decomposed by bacteria it gives organic acids plus phenol. Since these tiny larvæ could not take any plant protein but had to subsist on the amount of protein laid down in the egg mass it is possible that as soon as traces were formed in the digestive tract it was further decomposed. In dead insects this amino-acid is found (Muttowski). Cholesterol was also absent, this is found in all animal cells and especially in brain cells and nervous tissue. Egg oil, prepared from the yolk of eggs, contains always a certain percentage. I was able to demonstrate a trace in freshly laid eggs, but it disappeared rapidly. Cystine is necessary in food. It readily oxidizes itself and resembles the respiration processes. Nearly all proteins contain sulfur in small amounts in an unoxidized form (as sulfide sulfur). It occurs in the protein molecule either as cystine or cysteine and possibly in other amino-acids. This protein sulfur is oxidized in the body to sulfates and excreted as such. (Adult humans excrete about 2.5 grams sulfuric acid a day.) The larvæ in the egg cases must deposit these sulfates in the egg cases. Analytical figures on total sulfates will be given in the next paper of this series, suffice it to say that remarkable fluctuations occurred.

Albuminoid, simple proteins like collagen, according to Hawk (2) "include the principal organic constituents of the skeletal structure of animals as well as their external covering and its appendages." A glance at the table shows that almost twice as much albuminoid-nitrogen was present in the cover than in the total egg mass. The cover in this case constituted 48 per cent. of the total dry matter. Muttowski (5) demonstrated the presence of gelatine in insect blood. Although gelatine is a transformation product of collagen we will assume that all the albuminoid-nitrogen found was derived from gelatine. Gelatine contains 17.9 per cent. N and on the above assumption we found about 0.5 per cent. gelatine present in the cover. Gelatine upon hydrolysis yields amino-acids and it is possible that some amino-acids came from this source. However, cystine and tryptophane are not mentioned among the decomposition products of gelatine but these amino-acids were found to be present in the cover. We can say then that the cover is possibly for a small part made up of gelatine, mostly of simple proteins and another small part of carbohydrates (glycogen). Carbohydrates were present in comparatively considerable quantities in the larvæ confined to the egg cases and also some in the cover. Carbohydrates have the same tendency as fats, namely they have a ''protein sparing function'' in metabolism. That the carbohydrates were used is shown by the fact that sugars were present in the larvæ and not in the covers. The presence of carbohydrates in the cover might be explained on the basis that acetic acid is formed by hydrolysis of certain materials (mucin, chitin, etc.), aside from the presence of glycogen. Glycogen, animal starch, is an important constituent of muscle. The amount of this polysaccharide in muscle varies with muscular activity. Embryonic structures do not contain large amounts of glycogen. Vaney and Maignon (9) found a rapid increase in glycogen in the growing larvæ, reaching its maximum when the larvæ were full grown and decreasing continuously during pupation. This question will be dealt with in a later paper. From the table it can be seen that it was present in the larvæ and in the cover. The presence of phosphorus indicates that certain lipoids were present. This could be expected since one of the two great groups of tissue colloids is constituted of these substances, the other being proteins. It has been pointed out above that no cholesterol was present and it seems likely that the phosphorus present came mainly from phosphorized fats. The most important of the phosphorized fat group is lecithin which yield upon decomposition fatty acids, glycericphosphoric acid and choline.

Summary

Analyses of egg masses, larvæ, pupæ and adults of the tent caterpillar show that total nitrogen content of the egg masses from the time they were deposited to the hatching of the young larvæ was on an average of 13 per cent. There was an initial increase,

followed by a decrease and again followed by an increase. The average nitrogen content of the growing larvæ was about 10.2 per cent., decreasing from the time of hatching to the last instar. Nitrogen decreased considerably from the time the larvæ was full grown until the larvæ had "just pupated" (propupal stage) while it increased during the chrysalis stage. Adults again contained less nitrogen than the pupa in the last stage.

The relation between nitrogen and moisture and nitrogen and fatty substances is shown.

Analyses (total nitrogen, fats, ammonia-nitrogen, albuminoid nitrogen, amino-acids, glycogen and sugars) of the egg masses and nitrogenous cover of the egg cases are made a basis of discussion regarding the vital phenomena and decomposition processes of the larvæ confined to the egg cases.

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